3D genome alterations and editing in pathology

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Highlights

- Genome is folded into a multi-level 3D structure that controls many nuclear functions, including gene expression
- Alterations in 3D genome organization are linked to genetic diseases and cancer
- Several approaches have been developed to modify the global 3D genome organization and that of specific loci.
- Emerging experimental approaches of 3D genome editing may prove useful in biomedicine

Abbreviations

ABA - abscisic acid

ADLD - autosomal-dominant adult-onset demyelinating leukodystrophy

adRP - autosomal-dominant retinitis pigmentosa

AML - acute myeloid leukemia

BOFS - branchiooculofacial syndrome

CBS - CTCF binding site

CLOuD9 - chromatin loop reorganization using CRISPR-dCas9

CNS - central nervous system

CT - chromosome territory

FSHD - facioscapulohumeral muscular dystrophy

LAD - lamina-associated domain

LADL - light-activated dynamic looping

LCR - locus control region

NAD - nucleolus-associated domain

STR - short tandem repeats

SV - structural variation

T-ALL - T-cell acute lymphoid leukemia

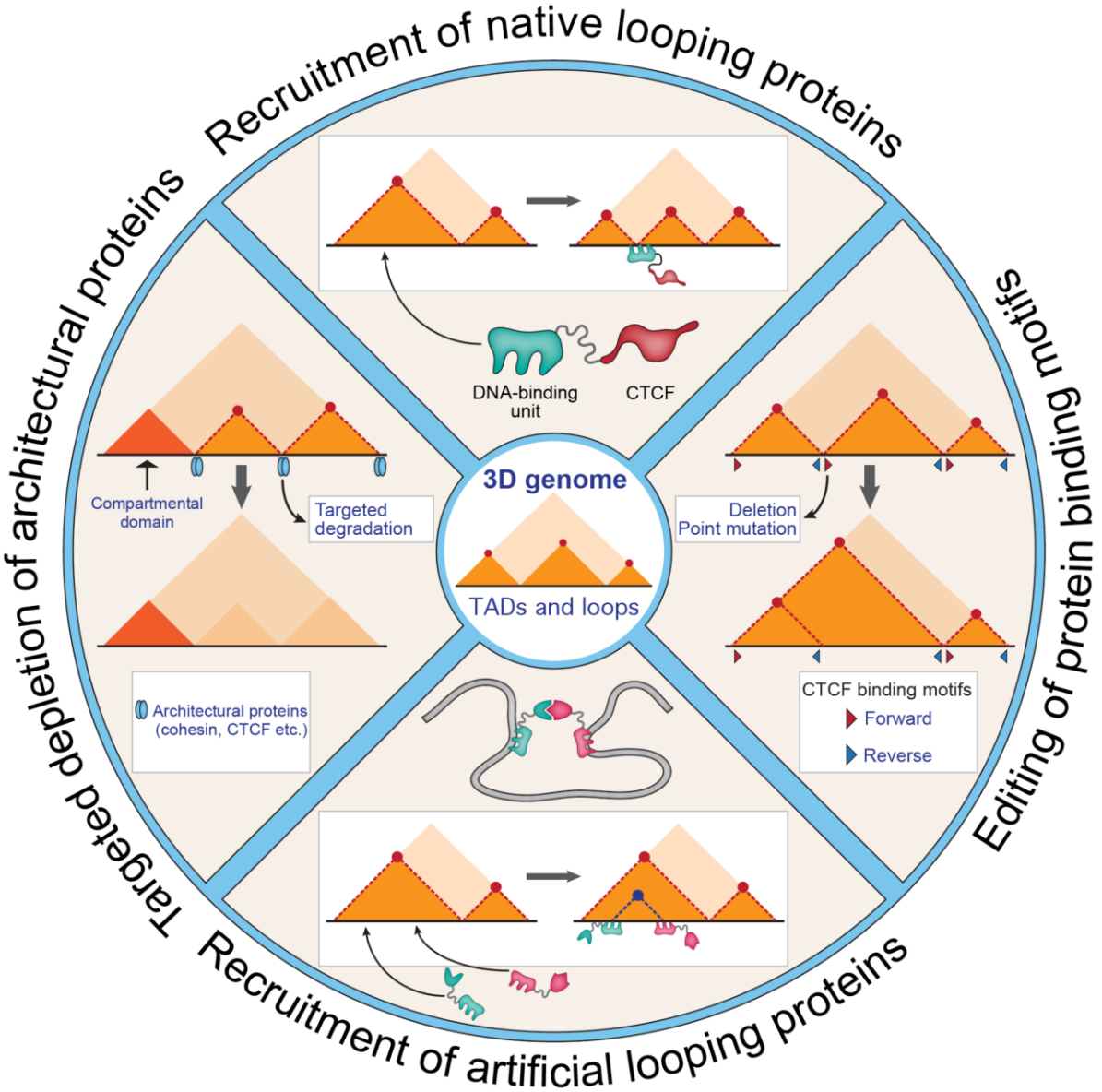
TAD - topologically associating domain

TALE - transcription activator-like effector

TF - transcription factor

ZFs - zinc finger nucleases

Graphical Absrtact



Graphical Abstract. Current approaches to 3D genome editing

ABSTRACT

Human genome is folded into a multi-level 3D structure that controls many nuclear functions, including gene expression. Recently, alterations in 3D genome organization were associated with several genetic diseases and cancer. As a consequence, experimental approaches are now developed to modify the global 3D genome organization and that of specific loci. Here we discuss emerging experimental approaches of 3D genome editing that may prove useful in biomedicine.

The overall 3D genome organization

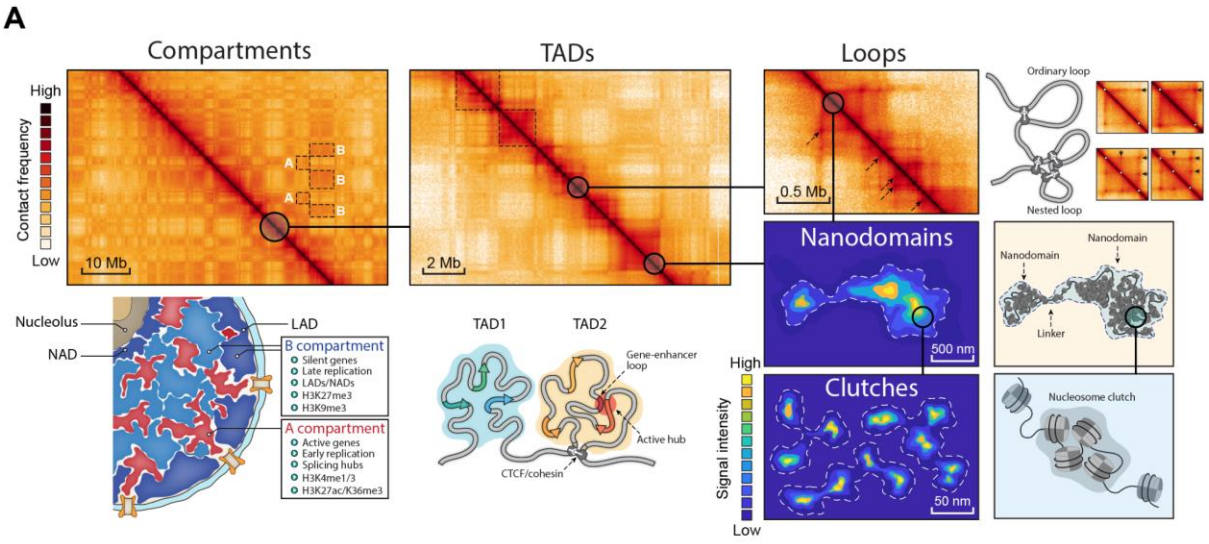
The interphase genome is folded in a highly ordered manner, essential both for DNA compaction and for the regulation of various intranuclear processes. Each chromosome occupies a restricted volume in the nucleus, a chromosome territory (CT). In mammals, large chromosomes and chromosomes with low gene density tend to localise at the nuclear periphery, whereas smaller chromosomes with high gene density are located more centrally (Boyle et al., 2001; Crosetto and Bienko, 2020). CTs have a spongy internal structure and are composed of bulk chromatin masses penetrated with the channels of the "interchromatin compartment", a dynamically organized system of cavities serving for the diffusion of nucleoplasm components (Albiez et al., 2006).

Within CTs, active and repressed genomic regions are spatially segregated into A and B compartments, respectively, formed by local *cis* as well as distant *cis* and *trans* interactions (Figure 1A) (Lieberman-Aiden et al., 2009). *A compartments* are early-replicating gene-rich and typically highly transcribed regions enriched in active histone marks such as H3K36me3, H3K27ac and H3K4me1. In contrast, *B compartments* contain late-replicating transcriptionally silenced regions enriched with nucleolus- and lamina-associated domains (NaDs and LADs, respectively) marked with H3K9me2 and H3K9me3 (Dillinger et al., 2017; Guelen et al., 2008; Nichols and Corces, 2021; Rao et al., 2014). Compartment partitioning strongly correlates with the transcription profile and thus is highly cell type-specific (Dixon et

al., 2015; Winick-Ng et al., 2021), whereas compartmentalization degree might vary significantly within a cell population (Bintu et al., 2018; Kim et al., 2020).

The compartments are further divided into subcompartments, distinguished by the patterns of different histone modifications and further into topologically-associated domains (TADs) with a high CTCF/cohesin occupancy at their borders (Gómez-Marín et al., 2015; Merkenschlager and Nora, 2016) and representing globular structures with a remarkable cell-to-cell variability in their 3D shape and folding density (Stevens et al., 2017). TADs serve as “warehouses” for genes, gene loci and their regulatory systems (Razin et al., 2016) delimiting the areas of enhancer action (Nora et al., 2017). Consequently, genes within a TAD are often co-regulated (Dixon et al., 2016); this is achieved by looping between enhancers/locus control regions and promoters (Amândio et al., 2020; Bonev et al., 2017). Molecular details of the loop formation mechanisms are still not fully understood, but cohesin-driven extrusion (Bauer et al., 2021; Davidson et al., 2019) and liquid-liquid phase separation (Hnisz et al., 2017; Razin and Gavrilov, 2020) are the most consistent models. The CTCF-cohesin complex preferentially determines strong long-range interactions including contacts between TAD borders (Rao et al., 2014), while the short-range enhancer-promoter and promoter-promoter interactions are also maintained through Mediator complex and various transcription factors (TFs) (Krietenstein et al., 2020) in cooperation with transcription machinery (Hsieh et al., 2020). Complex contact patterns within TADs are manifested in non-structured hierarchical nucleosome assemblies such as clutches (Ricci et al., 2015) and nanodomains (Szabo et al., 2018). Clutches are relatively small nucleosome agglomerates (about 2-20 nucleosomes/clutch) whose density and size strongly depend on the level of histone acetylation. This implies that clutches are formed by weak transient electrostatic interactions between nucleosomes. A group of clutches constitutes a nanodomain. Nanodomains are distributed throughout the nucleus, but their concentration increases near the nuclear periphery. Nanodomain structures are preserved upon CTCF and cohesin degradation and

they seem to be formed through liquid-liquid phase separation (Szabo et al., 2020; Ulianov et al., 2021).



B

Structure	Size and assay	Putative functions	Editing strategy
	Identified by C-methods		
Compartments	0.5-10 Mb, Hi-C	Spatial segregation of active and inactive chromatin	Unknown
TADs	0.1-1 Mb, Hi-C, 5C	Spatial insulation of neighboring genome loci and their regulatory systems	Mutations and rearrangements of CBSs
Loops	50-500 kb, Hi-C, 4C, Capture-C	Spatial insulation of neighboring genome loci and contacts between regulatory regions	Same as for TADs + CTCF recruitment
	Identified by microscopy		
Nanodomains	0.007-0.2 mkm^2, Oligopaint-FISH	Unknown	Unknown
Clutches	100-1000 nm^2, ICH, Chrom-EMT	Unknown	Unknown

Figure 1. Different levels of 3D genome organization. A, Upper panel: contact maps of 3D genome structures, lower panel: graphical representation; B, summary of different levels of 3D genome organization.

In sum, TADs represent cornerstone structural and functional units of the 3D genome. Below we highlight the role of TAD rearrangement and disruption of TAD borders in the development of severe pathologies, and discuss 3D genome editing techniques..

3D organization of the genome and pathologies

Over several past years, a number of pathologies have been associated with 3D genome structure abnormalities. Changes in loop and TAD profiles can be due to both genetic and epigenetic factors. The most common are SV (structural variation) mutations: rearrangements of 50 nucleotides or more in length such as deletions, duplications, insertions and translocations. SV mutations affecting the 3D genome in various diseases modulate promoter-enhancer interactions and affect gene expression (Nieboer and de Ridder, 2020; Zhang et al., 2021). They can change the proximity or accessibility of affected genomic regions, increasing the risks for certain chromosomal aberrations (Yang et al., 2022).

CTCF binding sites (CBSs) serve as barriers for cohesin-driven DNA-loop extrusion, the major determinant of a TAD profile in mammals (Nuebler et al., 2018). DNA methylation suppresses CTCF binding; thus allowing aberrant enhancer-promoter interactions that may affect disease-related genes. As we discuss below, this effect is common to several human diseases

In human glioma, mutations in the *IDH* gene result in an increased genome-wide level of DNA methylation including that in the *PDGFRA* oncogene locus. Methylation of the 5'-flanking insulator serving as a TAD boundary in this locus results in loss of CTCF binding and perturbs local interaction patterns that drives an abnormal activation of the *PDGFRA* gene by a distal enhancer (Flavahan et al., 2016).

In human 3p21.2 locus, the SV rs2535629 have a strong association with schizophrenia. rs2535629 (A/G) is located within the CBS which resides in a repressor element in the 7th intron of the ITIH3 gene. The presence of this structural variation prevents CTCF binding to CBS, and also changes the expression profiles of nearby genes: downregulates *GLT8D1* and *SFMBT1*, and upregulates *NEK4*. Downregulation of *SFMBT1* gene product involved in the regulation of proliferation and differentiation of nerve stem cells, as well as in the

formation of dendritic spines and the proper functioning of neural synapse, is associated with schizophrenia development (Li et al., 2022).

SVs within enhancers interacting with *MYH7* and *LMNA* can trigger the development of cardiomyopathy. *MYH7* and *LMNA* promoters establish spatial contacts with multiple enhancers, sometimes in a clustered manner. Many of these enhancers contain binding sites of various TFs including CTCF. Several SNPs (rs7149564, rs116554832 or rs10873105) suppress TF binding to DNA, probably affecting enhancer-promoter loops. For example, the SNP rs875908, 2 kb away from *MYH7*, destroys the TBX5 binding motif and is associated with a decrease in the thickness of the left ventricle in dilated cardiomyopathy (Gacita et al., 2021).

A myopathy associated with a SV affecting CBS is facioscapulohumeral muscular dystrophy (FSHD). This disease is related to the abnormal expression of the *DUX4* gene and other genes, such as *FRG1* and *FRG2*, have been proposed to contribute to FSHD pathology. *E*nhancers regulating *DUX4* and *FRG1* and their target genes are physically separated into two different loops due to the presence of an FR-MAR insulator (Himeda et al., 2014; Petrov et al., 2006, 2008). The insulator activity of FR-MAR is decreased by the presence of a certain SSLP and specific CpG methylation within the region in FSHD myoblasts (Kisseljova et al., 2014; Petrov et al., 2008).

In T-cell acute lymphoid leukaemia (T-ALL), multiple changes in gene activity and the profile of CTCF binding to DNA coincide within specific TADs, with a major impact on carcinogenesis-related regions in *NOTCH1* target genes (such as *APCDD1*, *IKZF2*, *CYLD* and *MYC* (Kloetgen et al., 2020). Disappearance of the TAD boundary between *MYC* and its superenhancer in T-ALL cells leads to Myc overexpression (Kloetgen et al., 2020). In this case, loss of CTCF binding is not caused by CBS mutations or methylation and is accompanied with the decrease of chromatin accessibility. This exemplifies that some additional factors may influence CTCF binding. One potential candidate in Jpx non-coding RNA that regulates CTCF binding to a subset of developmentally sensitive loci by competitive inhibition (Oh et al., 2021a).

Expansion of repeats and viral DNA integration could also affect the profile of spatial interactions between remote genomic elements. Short tandem repeats (STRs) alter TAD boundaries by modulating CTCF binding (Ibrahim and Mundlos, 2020). In several pathological models, STRs accumulate at TAD boundaries increasing the density of CpG islands which are often hypermethylated in pathologies. One example is the *FMR1* gene, whose repression leads to the fragile X chromosome syndrome (Martin-Bell syndrome). STR accumulation at the boundary of encompassing TAD promotes DNA hypermethylation followed by the decrease of CTCF binding and significant alteration in the enhancer landscape of the locus. These lead to the loss of the TAD boundary and *FMR1* repression (Sun et al., 2018).

Integration of primate-specific endogenous retrotransposon human endogenous retrovirus subfamily H (HERV-H) was shown to create TAD boundaries in the genome of human pluripotent stem cells (Zhang et al., 2019). In this case, active viral transcription, but not CBS within the viral genome, creates a TAD boundary at the site of the HERV-H integration. This is in line with recent observations showing that active transcription constitutes a barrier for the cohesin-driven extrusion (Brandão et al., 2019). In contrast to HERV-H, insertion of the human T-lymphotropic virus HTLV-1 establishes *de novo* loops due to the presence of CBS within the viral genome. This results in abnormal host gene transcription not only in loci proximal to the integration site, but also more than 300 kb away (Melamed et al., 2018). The same was observed for the bovine leukaemia virus (BLV) carrying several CBSs which were involved in formation of new chromatin loops with the host chromosome loci after the provirus integration (Bellefroid et al., 2022).

In some cases, violations of CTCF-dependent loop structures could be driven by external factors, such as drug use. For example, cocaine addiction results in a pathogenic looping

within the *IRXA* locus in brain neurons due to DNA hypomethylation at a set of CBSs (Vaillancourt et al., 2021).

Development of some diseases (in particular, cancer) is driven by changes in enhancer-promoter loops established by specific TFs. In melanoma, treatment with a BRAF inhibitor PLX4032 leads to survival and further expansion of cancer cells due to upregulation of the *MET* gene. *MET* is normally expressed in many tissues and cell lines, but treatment of melanoma cells with PLX4032 results in a seven-fold increase of *MET* expression that relies on the activity of a tissue-specific TF MITF. This factor is essential for the spatial contacts between the *MET* promoter and the enhancer located 63 kb away. This interaction triggers the *MET* upregulation and the HGF-MET signalling cascade. MITF depletion, as well as the destruction of its binding site in the *MET* enhancer, restores the sensitivity of melanoma cells to treatment with BRAF inhibitors (Webster et al., 2014). Changes in the expression of *VEGFA, MGA1, ZFAND3, TCF7L2, OPEN, GLIS3* and others genes due to disruption of their contacts with the enhancer/enhancer hub may contribute to the development of type 2 diabetes (Miguel-Escalada et al., 2019).

In T-ALL, extended deletions eliminating CTCF-bound topological borders result in aberrant activation of proto-oncogenes *TAL1* and *LMO2* by distal enhancers from adjacent genome loci (Hnisz et al., 2016).

Disruption of the TAD borders also occurs in Autosomal-dominant adult-onset demyelinating leukodystrophy (ADLD), a disorder characterised by a progressive demyelination of the central nervous system due to cerebral lamin B1 (*LMNB1)* overexpression. A 600-kb deletion eliminating a TAD boundary results in interactions between the three strong enhancers and the *LMNB1* promoter, causing *LMNB1* overexpression and myelin degeneration. In human 6p22.3 locus, deletion of an entire TAD including boundaries, correlated with activation of the *ID4* gene by enhancers from the neighbouring TAD in the

developing limb bud resulted in development of mesomelic dysplasia with hypoplastic tibia and fibula (Flöttmann et al., 2015).

SVs associated with changes in spatial genomic interactions also cause the Liebenberg syndrome, a limb malformation due to dysregulation of the *PITX1* expression: the forelimbs develop into the hindlimbs. *PITX1* controls the normal development of the hindlimbs where its expression is governed by the interaction with the *Pen* enhancer. In the forelimb buds, *PITX1* is not expressed due to spatial isolation from the *Pen* by the insulator located in the promoter region of the nearby *H2afy* gene. In Liebenberg syndrome, multiple deletions eliminate this insulator allowing the *Pen* enhancer to activate *PITX1* leading to abnormal formation of the forelimb bones and the kneecap near the elbow (Kragesteen et al., 2018, 2019).

Duplications can also affect the TAD profile and/or intra-TAD spatial interactions. In the *SOX9/KCNJ2/KCNJ16* locus, where genes encoding the developmetal regulator *SOX9* and potassium channels *KCNJ2/KCNJ16* are located in adjacent TADs, duplications within the *SOX9* TAD result in female to male sex reversal. These duplications encompassing the TAD boundary and the entire *KCNJ2* gene result in the formation of a new TAD, where *KCNJ2* is upregulated by SOX2- specific enhancers. This leads to the limb malformations with aplasia of nails and short digits known as Cooks syndrome (Franke et al., 2016). In addition to duplications, a translocation involving the *SOX9/KCNJ2/KCNJ16* locus leads to the Snijders Blok-Campeau syndrome characterized by intellectual disability, speech problems, and distinctive facial features (Melo et al., 2020).

Numerous SVs lead to the emergence of new TADs within the *YPEL2/LINC01476* locus leading to dysregulation of gene expression manifested in autosomal-dominant retinitis pigmentosa (adRP) (de Bruijn et al., 2020). In some loci, large-scale SVs cause a variety of distinct pathologies. The canonical example is the *WNT6/IHH/EPHA4/PAX3* locus where different deletions, inversions and duplications result in limb malformation. Some of the

intergenic SVs induce the F syndrome (acropectorovertebral dysgenesis), a rare inherited skeletal disorder characterized by the fusion of the thumb and index finger. In particular, the disease is caused by an inversion in the *EPHA4* gene locus, which leaves the TAD boundary intact, but places an enhancer from the neighbouring TAD near the *WNT6* gene promoting its overexpression. Other duplications and deletions affecting TAD structure within the regions cause brachydactyly and polydactyly (Lupiáñez et al., 2015).

Large-scale inversions lead to the branchiooculofacial syndrome (BOFS), characterised by skin, face and eye defects of varying severity. This pathology occurs when the normal expression of the neural crest regulator *TFAP2A* is impaired. Usually, it is a result of a partial gene deletion, however, a recent study (Laugsch et al., 2019), describes a patient with a 89-Mb inversion that does not affect *TFAP2A per se*, but compromises its expression. This inversion disrupts the original *TFAP2A*-containing TADs and the interaction between the *TFAP2A* gene and a group of its enhancers.

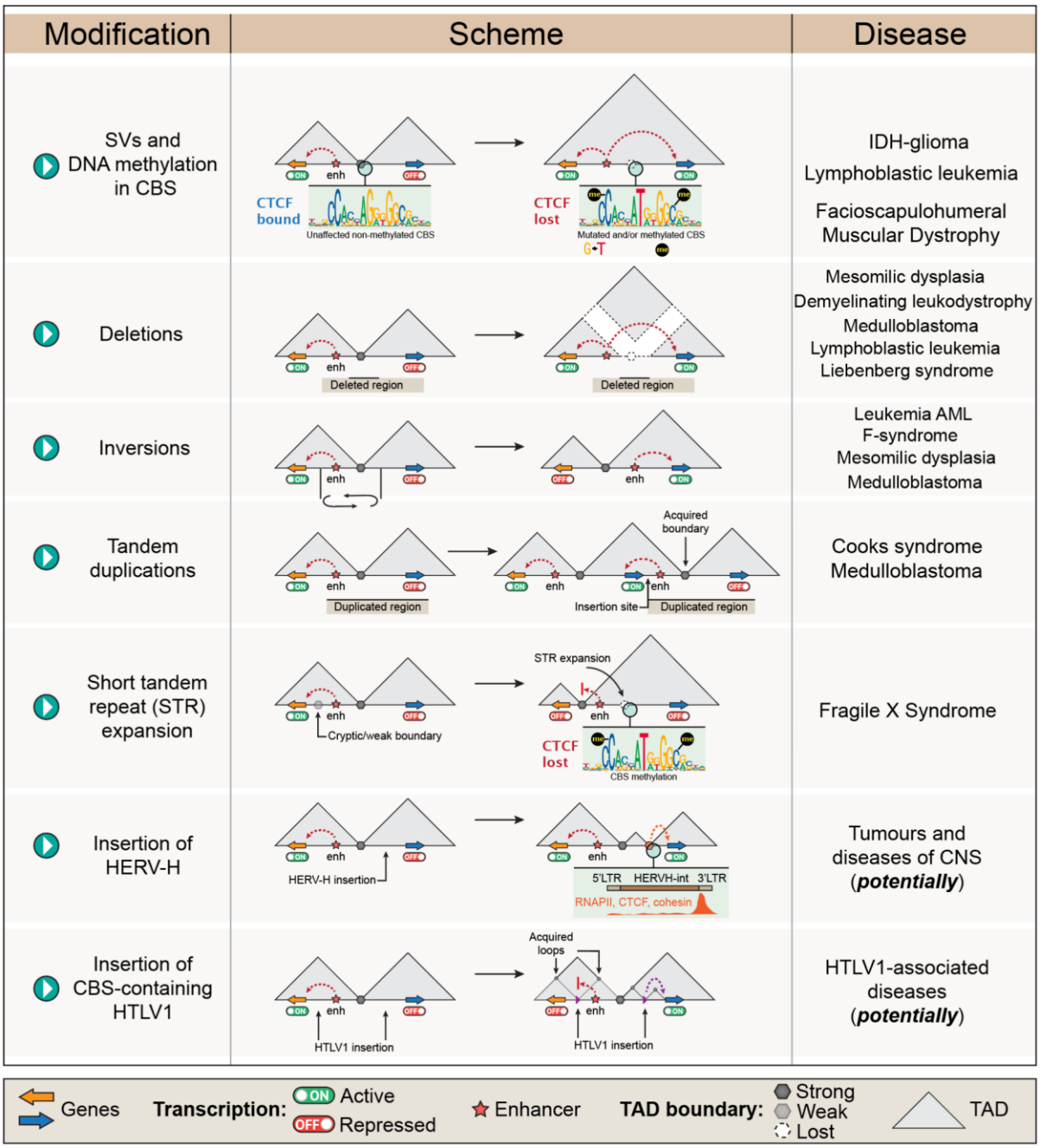


Figure 2. TAD-centric view on the 3D genome organization and disease

Finally, at a whole-nucleus scale, contacts between non-homologous chromosomes can also lead to oncogenic chromosomal translocations. This disrupts local regulatory 3D interaction networks and alters cellular transcription programs. Few examples include interactions of chromosomes 8 and 14 or chromosomes 11 and 14 in B-lymphocytes leading to Burkitt or mantle cell lymphoma, respectively; chromosomes 12 and 16 in adipocytes leading to t(12;16) in liposarcoma; chromosomes 5 and 6 in hepatocytes leading to t(5;6) in

hepatocarcinoma (Allinne et al., 2014; Germini et al., 2017; Kuroda et al., 2004; Parada et al., 2004; Roix et al., 2003).

Changes in 3D genome organization due to DNA methylation or SV mutations affecting 3D genome can lead to a phenomenon called “enhancer hijacking” when enhancers change their target, leading to gene expression abnormalities and pathologies. For example, a SV in the vicinity of the *GFI1B* gene leads to *GFI1B* interaction with distal superenhancers and overexpression in medulloblastoma (Northcott et al., 2014). Another example of enhancer hijacking associated with rearrangements involving loci encoding the *EVI1* stem-cell regulator and *GATA2.* In particular, inversion/translocation inv(3)(q21;q26.2) and t(3;3)(q21;q26.2) place the *GATA2* enhancer in a close proximity to the *EVI1* promoter . resulting in *EVI1* overexpression and GATA2 functional haploinsufficiency in acute myeloid leukemia (AML) cells (Gröschel et al., 2014).

Together, alterations in the genome 3D organization are quite common in cancer and developmental disorders. Screening revealed that 7.3% of balanced chromosomal abnormalities disrupt TADs at known syndromic loci (Redin et al., 2017), and 14% SVs affecting TAD boundaries in cancers lead to remarkable changes in expression of nearby genes (Akdemir et al., 2020). The ability of naturally occurring SVs to target 3D genome and cause socially-significant diseases imposes the development and further clinical validation of 3D genome editing technologies applicable for treating patients.

3D genome editing

Since alterations in the chromatin contact profiles are associated with many pathologies, development of the 3D genome editing methods is on the frontlines of biomedicine. The

existing approaches largely rely on the use of either native architectural proteins such as CTCF or artificial looping proteins.

As highlighted above, CTCF is a master regulator of the mammalian interphase genome folding (van Ruiten and Rowland, 2021), and thus is a predominant target for the 3D genome engineering. CBS deletion/insertion/inversion and point mutations is a streamlined path for the precise control of the CTCF binding within genome regulatory elements and TAD boundaries/loop anchors (Narendra et al., 2016; Willemin et al., 2021). In several loci, such manipulations alter loop profile and result in changes of gene expression that could be potentially used as a strategy for the cell type-specific transcription reprogramming in patients (Gong et al., 2019). However, genomic DNA editing possesses risks of chromosome rearrangements that should be considered upon the design of clinically-relevant applications (Samuelson et al., 2021).

In case of *de novo* TAD formation or excessive CTCF binding, depletion of CTCF from particular sites in chromatin can be accomplished through epigenetic modifications of CBS to inhibit CTCF binding without changes in the primary DNA sequence. In this case, chimeric proteins consisting of catalytically dead Cas9 (dCas9) fused with the Krüppel-associated box (KRAB), a H3K9me3-catalyzing repressor (dCas9-KRAB) or DNA-methyltransferases DNMT3A and DNMT3AL were shown to be effective inhibitors of CTCF binding when precisely targeted to the CBS (Liu et al., 2016; Tarjan et al., 2019).

Another possibility that is developed for potential clinical applications is the usage of small molecules interfering with the CTCF binding. Cell treatment with the anti-cancer agent curaxin CBL0137 leads to a partial depletion of CTCF from chromatin and compromises enhancer-promoter contacts (Kantidze et al., 2019). This opens an avenue for the rational *in silico* design of drugs acting as inhibitors of DNA binding and competitors for the protein-protein interactions for the major players in the 3D genome maintenance.

Since some diseases are associated with the decrease of CTCF occupancy at particular CBSs (Miyata et al., 2021), stabilisation of CTCF binding at such loci is a strategy to be considered. Several post-translational modifications are essential for the CTCF insulator and barrier activities (Pavlaki et al., 2018). Indeed, mutations in the CTCF region subjected to poly(ADP-ribosyl)ation by PARP1 compromise cohesin enrichment at CBSs, i.e. interfere with cohesin/CTCF-dependent loop formation (Pugacheva et al., 2020). In the Epstein Barr virus genome, PARP1 acts to stabilize CTCF binding at particular sites (Lupey-Green et al., 2018). Thus, inducible recruitment of PARP1 to certain CBSs could be a tool for the stabilization of selective CTCF binding to these sites. Other post-translational modifications of CTCF such as SUMOylation and phosphorylation (Kitchen and Schoenherr, 2010; Luo et al., 2020) are also of interest for the 3D genome manipulation in both genome-wide and locus-specific manner. Together, these examples illustrate that epigenome targeting and inducible CTCF post-translational modifications could potentially serve as a proxy for the 3D genome editing.

A number of diseases are characterized by a complete loss of CBSs at critical regulatory elements due to deletions and other SVs (Dahlqvist et al., 2021; Guo et al., 2018; Ushiki et al., 2021). In such cases, recruitment of CTCF by an unrelated DNA-binding module and restoration of the original loop profile might be a potential treatment strategy. One example is a dCas9-mediated CTCF recruitment enforced by the coupling with the SunTag technology allowing recruitment of multiple CTCF molecules to the same binding site (Oh et al., 2021b). This method has been tested in the *TFF* locus associated with breast, lung and colon cancers (Xu et al., 2013) and demonstrated a potential utility for the 3D genome engineering in multigene disease-associated loci.

Some pathologies are accompanied by a total loss of *CTCF* expression followed by genome-wide alterations of chromatin 3D structure. Consequently, insertion of a functional CTCF

gene could restore an original loop pattern of the affected loci. For example, breast cancer could be inhibited by the increased *CTCF* expression; the *CTCF* gene is frequently deleted in this type of cancer and this negatively affects the survival rate of patients at late stages (Duan et al., 2022). *CTCF* gene insertion by pseudoviruses also slows down the cancer cell divisions and/or migration, as well as metastasis in the lungs and brain, and affects the expression of almost 130 genes.

An alternative approach for manipulation of the 3D genome architecture relies on the expression of chimeric proteins containing DNA-binding modules (ZF, TALE, dCas9) fused with units forming homo- or heterodimers, such as dimerization domains of mammalian transcription factors, e.g. the self-associating (SA) domain of the Ldb1 protein (Deng et al., 2014). These chimeric proteins form relatively stable dimers and are suitable for the formation of constant distant interactions in chromatin. These contacts can be made reversible *via* inducible polymerization. One example is CLOuD9 chromatin loop reorganisation system where PYL1 and ABI1 fused with two different dCas modules interact with each other in the presence of abscisic acid (ABA). Application of the CLOuD9 system to the *Oct4* locus associated with various types of cancers demonstrated that recruitment of these fusion proteins to the *Oct4* promoter and its distal enhancer induced loop formation and upregulation of *Oct4* after ABA addition (Morgan et al., 2017). A similar approach, LADL (light-activated dynamic looping), utilizes co-expression of cryptochrome 2 (CRY2) and truncated CIB1 (CIBN) fused to dCas9. Exposure of cells to 470-nm blue light induces dimerization of CRY2, and heteromerization of CRY2 and CIBN. As a result, loci targeted by dCas9-CIBN form a transient loop. In a proof-of-concept study, LADL-induced looping between *Zfp462* and the *Klf4* superenhancer in mouse embryonic stem cells was successfully used to increase the *Zfp462* expression (Kim et al., 2019).

Concluding remarks

Genome-wide association studies revealed a number of genomic SVs associated with the development of various diseases. Most of these SVs are located outside genes and their regulatory modules. Consequently, the mechanical links between disease-associated SV and regulation of genome activity remained obscure. Recent results discussed here argue that many of the disease-associated SVs affect the 3D genome organization. This raises the issue of the need for 3D genome editing. Several approaches for such editing have been proposed and tested on cell cultures. The question is whether any of the developed strategies has the prospect of practical application in the treatment of patients. In the case of cancer, the straightforward strategy is to kill a cancer cell if it can be recognized and targeted rather than to try to correct anything in this cell. The only possible application here is to use low molecular weight agents (ex: curaxins) that affect all cells with some preference to cancer cells. More interesting opportunities for practical applications of 3D genome editing arise in cases where it is necessary to deliver to the organism its own normal cells, which will exist alongside corrupted ones or replace them. This strategy assumes that damaged cells are taken from the patient, manipulated in the laboratory and returned to the patient's body. In the future, this approach could be useful for the treatment of a number of diseases of the hematopoietic and endocrine organs, as well as some types of muscular dystrophies.

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